CONTRIBUTIONS TO THE STUDY OF PARASITIC PROTOZOA. II.*

MYXOBOLUS TOYAMAI NOV. SPEC., A NEW MYXOSPORIDIAN PARASITE IN CYPRINUS CARPIO L.

ROKUSABURO KUDO

While studying Cnidosporidia in some fresh-water fishes during the last few months, my attention was attracted to a minute white spot on the branchial lamella of a *Cyprinus carpio*. Examination under the microscope showed that the white spot was no other than a round cyst of a myxosporidian containing numbers of ripe spores each having only one polar capsule. The fish that harbored the Myxosporidia was a year old, having a length of about 6 cm. On searching carefully all the branchiae of the fish under the dissecting microscope, I found another round body situated near the free end of a branchial lamella, the diameter of which was about 200μ. Since that time, many fishes of the same kind, and reared in the same pond where the above-mentioned infected fish had been found, have been examined for the same parasite, but it has not been found again. Consequently, the material is too scanty for detailed study. I will try, however, in the following pages, to give the results of observations on the one fish, which are probably of some interest, since the morphology, and especially the life-history, of the unicapsulated Myxosporidia seem, so far as I am aware, to have been left in obscurity.

The branchiae of the infected fish were cut into pieces, fixed with Schaudinn's or Fleming's fluid, imbedded in paraffin, cut in serial sections of 2 to 4μ thickness and stained with Giemsa's solution or Heidenhain's iron hematoxylin, the latter being counterstained with eosin or orange G.

MORPHOLOGY OF THE TROPHIC STAGE

I expected that in sections would be found many young developmental phases of the organism which could not be observed externally in the fresh state, but in the study of the numerous sections, to my disappointment, only very few of the parasites were observed, showing that the infection in the present case was one of slight degree. The focus of infection was the connective tissue of the gill filament. The connective tissue became swollen by the infection of the parasite, and with its growth the tissue around it formed a thick layer, penetrated by numbers of capillaries (Figs. 1 to 3). A similar phenomenon has

* From the Pathological Laboratory of the Imperial Sericultural Experiment Station, Nakano, Tokio, Japan. Director, Dr. T. Kagayama.

already been observed by Cohn in *Myxobolus minutus* and by Schröder in *Henneguya acerinae*. The parasite in the branchiae is generally ovoidal in form (Fig. 1), but sometimes a calabash-shaped one is present (Fig. 2). This is probably caused by the union of two closely neighboring individuals. The gill-filament infected is not so greatly swollen as is the case with *Henneguya acerinae, Myxosoma dujardini* according to Thélohan (1895), and *Henneguya gigantea* according to Nemeczek.

The youngest form found has an oval shape. The dimensions are about 67 by 50μ, showing clearly the differentiation of the protoplasm into ectoplasm and endoplasm. The ectoplasm exhibits vertical striations (Fig. 3), similar to those of *Myxidium lieberkühni, Myxobolus pfeifferi* according to Thélohan (1895), of *Henneguya acerinae* and also of *Sphaeromyxa sabrazesi* according to Schröder (1907), and *Myxobolus gigas* according to Auerbach. Besides this structure, in some specimens the ectoplasm differentiates very fine plasmic processes, 2 to 3μ long, from its surface (Fig. 3). Auerbach (1909) noted a structure analogous to the above-mentioned one in *Myxobolus fuhrmanni,* but he could not determine whether it belonged to the parasite or to the tissue of the host. Schröder (1907) observed a similar differentiation of the ectoplasm in *Sphaeromyxa sabrazesi,* stating that "an der Oberfläche des Ektoplasms erkannte ich bei einigen Exemplaren einen zottenähnlichen, wenig über 1μ hohen Besatz."

The endoplasm has a coarsely granulated structure. The nuclei are round or oblong, in size varying from 1 to 4μ. They are scattered in the endoplasm, unlike the nuclei observed previously by Thélohan, Schröder, etc., who found them situated rather in the middle portion of the endoplasm.

In some young specimens, where the spore formation had begun to take place, I noticed that the nuclei and the pansporoblasts took a peripheral position, while in the middle portion a large round granulated body of distinct contour, but with no nucleus in it, was observed. I could not determine whether it is an accumulation of the endoplasm or an inclusion.

In the older cyst, which is oval-shaped and of about 190μ in maximum diameter, the ectoplasm becomes thinner than in the younger form. In the periphery of the endoplasm, numbers of nuclei are to be found, and towards the middle portion of it matured spores and several developmental phases of pansporoblasts to spores.

SPORE FORMATION

The nuclei in the plasmodium may be distinguished as vegetative and generative. The latter are always found in a round cell which takes stains more deeply than the surrounding endoplasm. The uninu-

cleate cells are the "sphères primitives" of Thélohan (1895), "pansporoblasts" of Gurley, or "Propagationszellen" of Keysselitz (1908). The propagative cell is of oblong or spindle shape, though usually round in form, with dimensions of 4 to 8μ. The nucleus is often situated excentrically (Fig. 4). A caryosom, as Keysselitz mentioned, is always found in it. The propagative cell multiplies by division into two or three daughter cells (Figs. 5 to 16). These points correspond to some extent with those of *Myxobolus pfeifferi* according to Keysselitz (1908) and to Mercier, and *Myxidium bergense* according to Auerbach (1912). The nuclear division in the propagative cell of *Sphaeromyxa sabrazesi* according to Schröder (1907 and 1910), *Myxobolus pfeifferi* according to Keysselitz and to Mercier, and *Henneguya psorospermica* according to Auerbach, is reported to be mitotic. In the present form I also observed mitotic division. The chromatin, through the coil stage (Figs. 5 to 7), divides into two parts, exhibiting very often the central spindle (Figs. 8 to 10). In this respect it resembles that of *Myxidium bergense* studied by Auerbach (1912).

The propagative cells resulting from the multiplication go on to spore formation. The greater propagative cell (macrogamete) and the smaller one (microgamete) take an elongated form and associate with their lateral surfaces. At first a space is seen between them (Figs. 17 and 18), and finally the cytoplasm of both cells fuses at the place of contact (Figs. 19 to 23).

The association of two binucleate cells, observed by Schröder in *Sphaeromyxa sabrazesi* and by Keysselitz (1908) in *Myxobolus peifferi,* does not exist in the present parasite. The association of the two uninculeate propagative cells in the present Myxosporidia strikingly resembles those observed by Mercier (1904) in *Myxobolus pfeifferi* and by Auerbach (1912) in *Myxidium bergense.* But the nuclei of the associated form do not fuse into one, as Mercier thought happened in *Myxobolus pfeifferi.*

The nuclear change in the pansporoblast coincides to some extent with that mentioned by Auerbach (1912) in *Myxidium bergense.* Instead of uniting into one, the nuclei in the associated form undergo division. The smaller nucleus divides into two at the peripheral position of the pansporoblast, being destined for the nuclei of the pansporoblast (Figs. 23 to 26). The greater nucleus repeatedly divides mitotically with the growth of the pansporoblast (Figs. 22, 25 to 31). In the fully developed pansporoblast, ten nuclei are observed, besides two nuclei of the pansporoblast and the reducing nuclei. At this stage, the contents of the pansporoblast separate into two sporoblasts, each of which contains five nuclei (Fig. 31). Of the five, two are found in a plasmic mass (sporoplasm in the later stage), one is in a cell which usually has a vacuole in it (nucleus for polar capsule and polar fila-

ment), and the remaining two are for the spore membrane. They are clearly recognizable in young spores, as is shown in Figures 32 to 37. When the spore is fully developed, the membrane of the pansporoblast is broken up, and the spores consequently become free in the endoplasm as in bicapsulated Myxobolus according to Keysselitz. As I mentioned above, we always recognize several developmental stages of the spore in the older cyst.

MORPHOLOGY OF THE SPORE

The spore has a pyriform shape, with a peculiar attenuated anterior and broadly rounded posterior extremity (Figs. 38 to 45). It has no bilateral symmetry. The spore-membranes of *lateral surfaces* are usually curved in opposite directions (Figs. 39 and 40). The form agrees well with that of *Myxobolus piriformis* described and illustrated by Balbiani and by Thélohan (1895). Spores of the calabash shape, however, occur not infrequently in the present case (Fig. 38). The spore-wall is comparatively thin and composed of two valves, superior and inferior. At the plane of junction the shell is somewhat thickened (Figs. 41 and 44). The surface of the spore usually represents no special structure. Very rarely a single, short, tail-like process about 1.5μ in length is seen at the middle part of the posterior end (Fig. 41). Thélohan (1895) observed a similar abnormality of the spore in *Myxosoma dujardini* and described that "quelques spores anormales ont un prolongement caudal." I also regard the above-mentioned process in certain spores as an abnormality. The length of the spore is about 15μ, the breadth 7 to 8μ and the thickness 5 to 6μ. Thélohan gives the size of the spore of *Myxobolus piriformis* to be 16 to 18 by 7 to 8μ. In the fresh preparations one pyriform polar capsule is observed at the anterior half portion of the spore (Figs. 42 to 44), its dimensions beings 7 to 8 by 3 to 4μ. The wall is drawn out anteriorly into a minute duct which pierces the shell near its anterior extremity, affording exit for the polar filament. Thélohan did not measure the size of the polar capsule of *Myxobolus piriformis*. But it seems to be much smaller than the present form (compare his Figures 116 and 117, Plate IX, 1895, with my Figures 38 to 45). Auerbach (1909) observes spores with two polar capsules among unicapsulated spores of *Myxobolus fuhrmanni*. In the present case, all spores have only one large polar capsule each, of which I will speak again when I come to the permanent preparations. Moreover, in some spores, the nucleus of the polar capsule is seen to be attached to it (Figs 42 and 43). The polar filament is easily extruded from the anterior end of the polar capsule when the spore is treated (Fig. 45) with a reagent like caustic potash, or hydroxyl, or pressed mechanically between the cover and slide glasses. The length of the filament measured after the spore has been

freshly prepared and pressed agrees usually with the measurements of stained ones prepared according to my method (1913). The length of the polar filament of the parasite is 40 to 45μ, so it is 10 to 15μ longer than that of *Myxobolus piriformis* measured by Thélohan. The posterior half portion of the spore is filled with sporoplasm. In fresh preparations, it is of a transparent, somewhat granular structure. Treated with iodin-alcohol, there appears a large vacuole stained brownish yellow in the sporoplasm.

In fixed preparations, the anterior end of the spore-membrane is stained very faintly (Figs. 38 to 41). The duct of the polar capsule becomes easily visible. In some spores, close to the anterior end of the polar capsule, there is an oblong mass of protoplasm (Figs. 38 to 40). I took this structure at first to be a polar capsule and compared it with the "Körperchen" of *Pimelodus blochii* of Müller, i. e., *Myxobolus inequalis* described by Gurley. But no such structure is observed in my present preparations, so that I cannot determine whether it is a degenerating polar capsule or some other structure. The nucleus of the polar capsule is always observed in young spores, well stained at the peripheral part of the capsulogenous cell (Figs. 33 to 37). In the sporoplasm, a large iodophile vacuole remains unstained, its diameter being about 3μ. The iodophile vacuole of *Myxobolus piriformis* observed by Thélohan is smaller than the present one. Two nuclei are always found in the sporoplasm situated closely to each other. They are usually of equal size (Figs. 37 to 41), but sometimes of different dimensions (Fig. 37). The position of the nuclei in the sporoplasm is not always the same. They are seen between the polar capsule and the vacuole (Fig. 39), on a *lateral aspect* of the vacuole (Fig. 38), or near to the posterior end of the spore (Figs. 40 and 41).

To what genus and what species does the present parasite belong? Because of the presence of an iodophile vacuole, it is clear that it belongs to the genus Myxobolus. So far as I am aware, the unicapsulated Myxosporidia known up to the present time are four in number, all belonging to the genus Myxobolus:

Myxobolus piriformis Thélohan
Myxobolus unicapsulatus Gurley
Myxobolus fuhrmanni Auerbach
Myxobolus oculi-leucisci Trojan

Of these, *Myxobolus unicapsulatus* is quite different from the present form. If one compares Figures 5, Plate 16 of Müller (1841) with my Figures 38 to 41, one sees great differences between the spores. Moreover, the habitat is quite different. *Myxobolus fuhrmanni* as stated by Auerbach (1909) was found in the connective tissue of the mouth of *Leuciscus rutilus* L. The spore is much larger than the present one

Explanation of Plates

All figures except Nos. 1 and 2 are drawn with Abbe's drawing camera.

Figs. 1 to 41 from sections.

Figs. 42 to 45 from fresh preparations.

Staining: Figs. 7, 9, 14, 17, 18, 21, 22 and 35: Giemsa's solution.

All the others: Heidenhain's iron hematoxylin and eosin.

PLATES I AND II

Figs. 1 and 2.—Parts of longitudinal sections of infected branchial lamellae, showing the seat of the parasite. 1, × 160; 2, × 320.

Fig. 3.—A peripheral portion of the parasite, showing the differentiation of the protoplasm. × 1000.

Fig. 4.—A propagative cell from the plasmodium. × 2250.

Figs. 5 to 16.—Division of the propagative cell. × 2250.

Figs. 17 to 21.—Association of the macro- and microgametes. × 2250.

Fig. 22.—Nuclear division of a macrogamete. × 2250.

Figs. 23 and 24.—The same of the microgamete. × 2250.

Figs. 25 to 30.—Several developmental stages of the pansporoblast. × 2250.

Figs. 31 and 32.—Segmentation of the pansporoblast into two sporoblasts. × 2250.

Figs. 33 to 37.—Young spores in development. × 2250.

Figs. 38 to 41. Matured spores. × 2250.

Figs. 42 to 45—Spores from fresh preparations. × 1000.

Fig. 45.—A spore with polar filament extruded. × 1000.

PLATE I

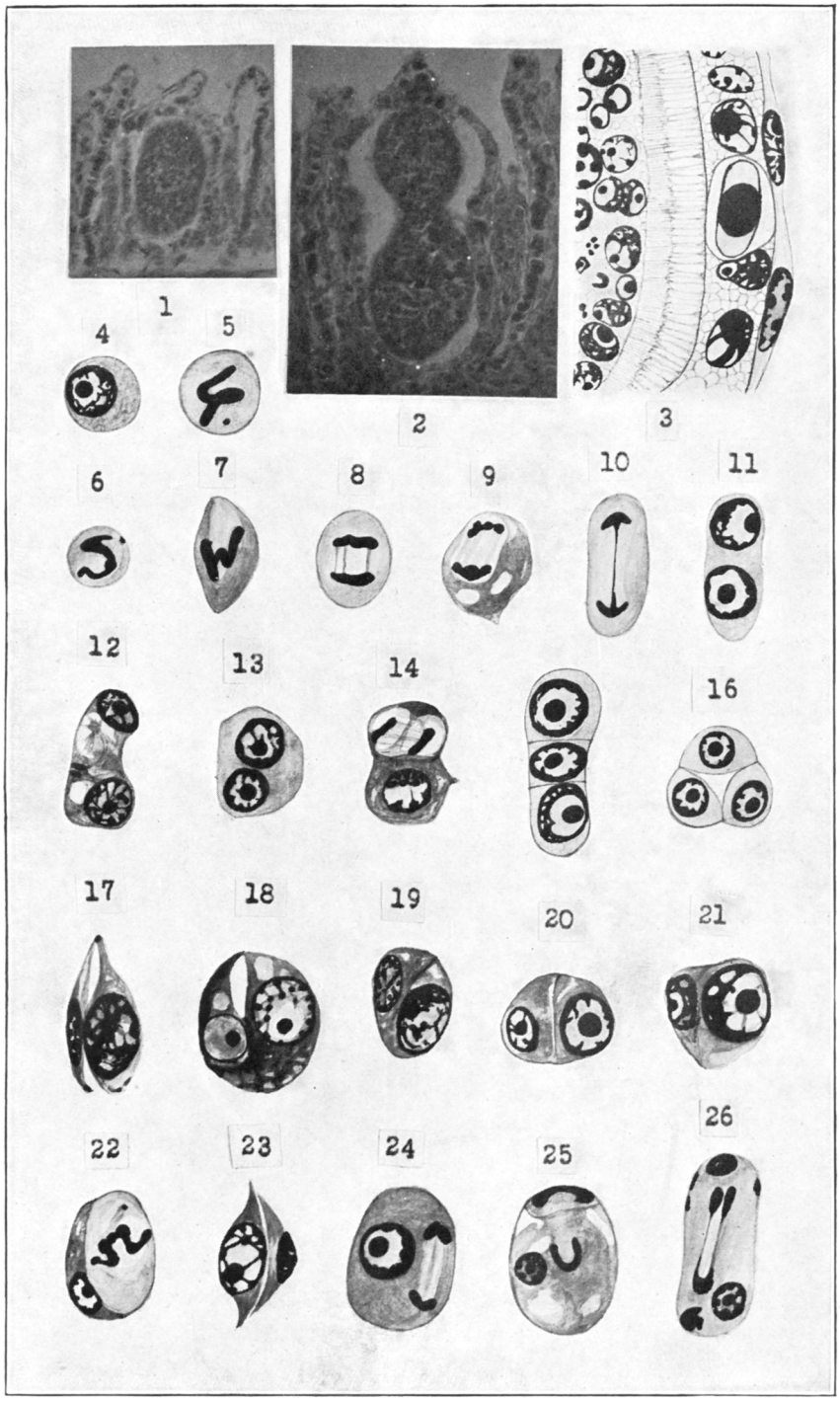
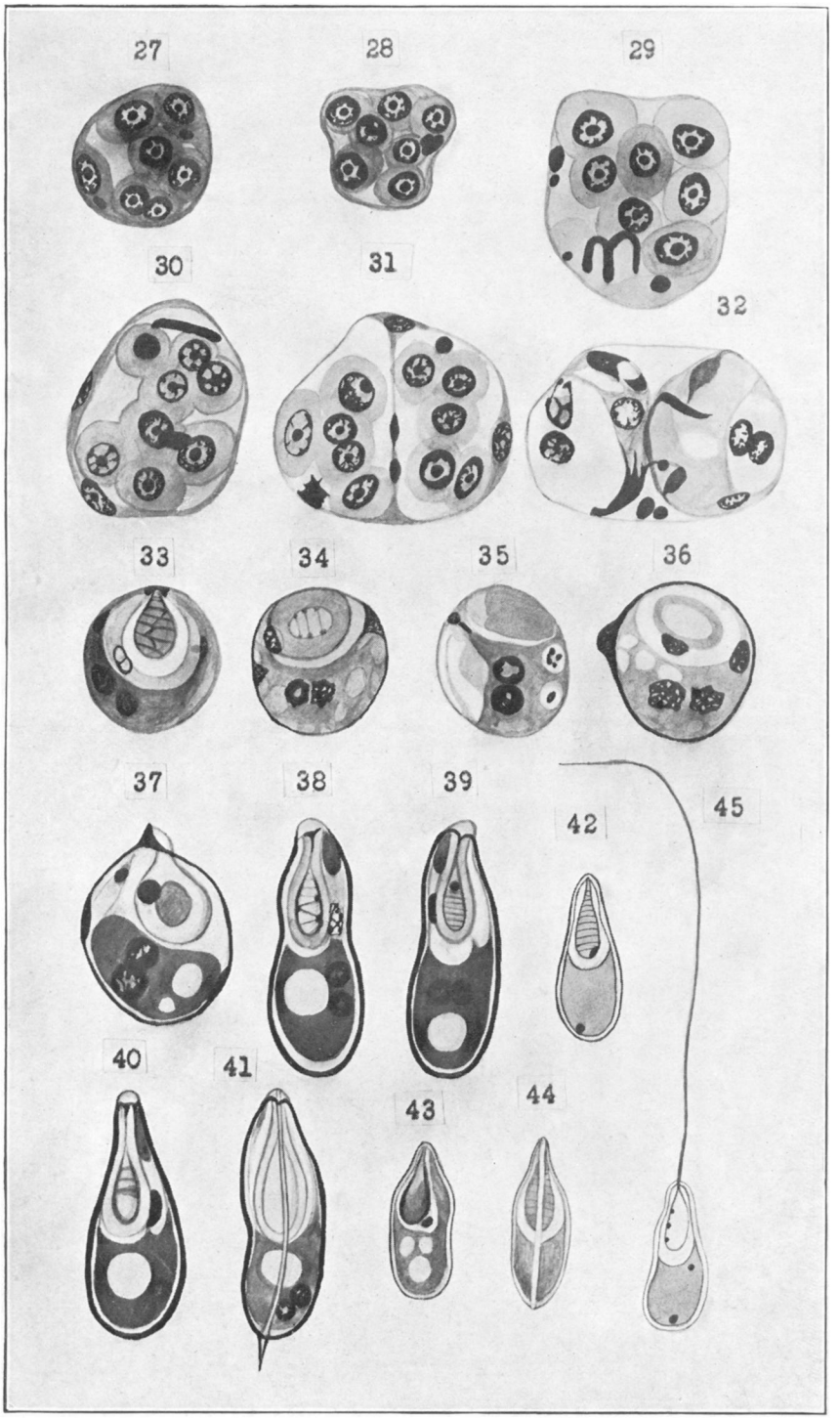


PLATE II



(length 18 to 20μ; breadth, about 8μ; thickness, 6μ, and the length of the polar capsule, 9 to 10μ). The spore membrane is thickened at the posterior end and has 4 to 6 notches. None of these points agree with the observations mentioned above on the present Myxobolus. The same is true of *Myxobolus oculi-leucisci,* which was found according to Trojan (1909) in the vitreous humor of the eye of *Leuciscus rutilus* L. Though the size of the cyst is almost equal to my parasite, the spore is smaller and different in structure.

I have spoken only partially of the comparison between the present Myxobolus and *Myxobolus piriformis,* and will compare them here again in the following synopsis:

	Myxobolus piriformis	The present Myxobolus
Habitat	Branchiae and spleen of *Tinca tinca* L.; kidney of *Misgurnus fossilis*	Branchiae of *Cyprinus carpio* L.
Cyst	"Les kystes branchiaux de cette espèce se reconnaissent à leur minceur: il forment de petites stries filiformes et non des tumeurs sphériques comme le *M. ellipsoides*" (Thélohan, 1895: 348)	Small round cyst in the connective tissue of the gill-filament
Spore:		
Form	Pyriform, with attenuated anterior extremity	Pyriform, with attenuated anterior end; often calabash form
Size	Length 16 to 18μ Breadth (Max.) 7 to 8μ	Length 15μ Breadth 7 to 8μ Thickness 5 to 6μ
Polar	Undescribed, figured only, capsule seems smaller	7 to 8μ by 3 to 4μ
Iodine vacuole	Smaller	About 3 to 4μ in diameter

As will be seen from the above comparison, there are great differences in the form of the cyst, the host, the size of the polar capsule, and the length of the polar filament, though the form and dimensions of the spore resemble each other.

Hence I think the Myxobolus found by me is a new species, and propose to call it *Myxobolus toyamai* nov. spec. in honor of Prof. Dr. K. Toyama, who kindly introduced me to this branch of protozoology in the year 1909.

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Note. This paper was printed in Japanese in 1915 and is reprinted here at the request of the author.